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EFFECT OF PROLONGED DEPOLARIZATIONS ON TWITCH TENSION AND INTRACELLULAR SODIUM ACTIVITY IN SHEEP CARDIAC PURKINJE FIBRES

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SUMMARY

1. Twitch tension and intracellular Na^+ activity (a^i_{Na}) were measured in voltage-clamped sheep cardiac Purkinje fibres. a^i_{Na} was measured using Na^+-sensitive micro-electrodes filled with the liquid ion exchange resin. ETH 227. The stimulus for contraction was a constant 200 ms depolarizing pulse to 0 mV from a holding potential of −80 mV delivered at 0·25 Hz. Prolonged test pulses for 1·8 s (post-pulses) were applied at the end of the stimulus pulse. The effects of post-pulses on twitch tension and a^i_{Na} were examined.

2. Post-pulses in the range of −40 mV reduced twitch tension below control force produced without post-pulse. Progressively more positive post-pulses to levels above 0 mV profoundly increased twitch tension, with a greater than 400 % rise in tension at +50 to +60 mV compared to control tension. a^i_{Na} declined at positive post-pulse potentials by more than 2 mM at +30 to +40 mV.

3. Tetrodotoxin (100 μM) did not affect the post-pulse voltage–tension or voltage–a^i_{Na} relation. Ca^{2+} channel modulation with nitrendipine (1 μM) similarly did not alter the post-pulse voltage–tension relation.

4. Removal of extracellular Na^+ eliminated the nadir in tension at post-pulses to −40 mV and the augmentation of tension at post-pulses above 0 mV.

5. We interpret these findings as evidence of voltage-sensitive Na–Ca exchange promoting net Ca^{2+} influx and net Na^+ efflux during positive post-pulses. The unusual shape of the post-pulse voltage–tension relation curve can be accounted for by a charged-carrier model of electrogenic Na–Ca exchange. The inverse relation between a^i_{Na} and twitch tension probably reflects the combined effects of reduced a^i_{Na} leak and changes in Na^+ and Ca^{2+} flux via voltage-sensitive Na–Ca exchange.

INTRODUCTION

The development of twitch tension in the heart is thought to be dependent on the phasic release of Ca^{2+} from the sarcoplasmic reticulum (s.r.) in response to the trans-sarcolemmal influx of Ca^{2+} (see Fabiato, 1983 for review). The strength of

contraction, therefore, is determined both by the supply of Ca^{2+} available for release from the s.r. and by the effectiveness of the trigger for Ca^{2+} release. Excitation–contraction coupling, or the influence of voltage on these processes, is only partially understood at present. The majority of attention has been focused on the effects of membrane potential on the voltage–sensitive Ca^{2+} channels through which the Ca^{2+} current flows. The Na–Ca exchange mechanism (Reuter & Seitz, 1968) has been assigned an indirect regulatory role that is linked to ionic concentration gradients. However, to the extent that Na–Ca exchange is voltage dependent, membrane potential should also influence tension via Na–Ca exchange, The aim of the present study is to dissect out some of the processes that control twitch tension and in particular to determine whether Na–Ca exchange exerts any voltage-dependent effects on force.

The effect of membrane potential on twitch tension reflects in part an effect on the triggering Ca^{2+} current stimulus (I_{si}). Analysis of Ca^{2+} currents in single cells has produced evidence that there are in fact two distinct currents, a fast inactivating current ($I_{Ca,f}$) which peaks at -40 to -30 mV (Lee, Noble, Lee & Spindler, 1984; Bean, 1985) and a slowly inactivating component ($I_{Ca,s}$) which may peak at $+10$ to $+20$ mV (Bean, 1985). A composite of these two currents probably forms the I_{si} that is detected in multicellular systems (see Noble, 1984, for review). It is possible that membrane potential may also modulate contractility by loading the s.r. with Ca^{2+} via either of these Ca^{2+} currents. However, this scheme describing excitation–contraction coupling is probably incomplete. Steady-state tension in voltage-clamped multicellular systems does not decline at increasingly positive clamp potentials above 0 mV as predicted by the current–voltage relation for the Ca^{2+} currents, but rather reaches a plateau (Beeler & Reuter, 1970) or continues to rise (Gibbons & Fozzard, 1975). These observations suggest that another mechanism aside from voltage-mediated Ca^{2+} currents may account for the continued rise in twitch tension up to clamp potentials of $+40$ mV.

The Na–Ca exchange mechanism may also load cells with Ca^{2+} during depolarizations to relatively positive potentials, if it were voltage sensitive with a stoicheiometry involving the exchange of more than two Na^+ for each Ca^{2+}. It is usually assumed that the predominant action of the Na–Ca exchange mechanism is to expel Ca^{2+} when the cell is near the resting potential. Using the Na–Ca equilibrium exchange equation (Mullins, 1979) or a charged-carrier model of Na–Ca exchange (Eisner & Lederer, 1985), one can predict that under steady-state conditions the counter-transport mechanism should extrude less Ca^{2+} or even promote trans-sarcolemmal Ca^{2+} influx at sufficiently positive potentials. If these assumptions regarding Na–Ca exchange are valid, then membrane potential and duration of depolarization may promote net intracellular Ca^{2+} gain during the action potential plateau or voltage-clamp pulses and thereby may alter contractility.

The present work examines the hypothesis that the amplitude and duration of changes in membrane potential govern twitch tension in part by effects on a voltage-dependent Na–Ca exchange mechanism. Accordingly, protocols were designed in voltage-clamped sheep Purkinje fibres that attempt to separate the effects of membrane potential on the stimulus for contraction (I_{si}) from voltage effects on Ca^{2+} loading (Na–Ca exchange or I_{Ca}). The voltage effect on the stimulus for

contraction was controlled by using standard pulses of constant duration and amplitude. Long voltage pulses to varying potentials (post-pulses) were appended to these standard pulses. The amplitudes of the post-pulses were found to have profound effects on steady-state twitch tension and intracellular Na^+ activity (a^i_{Na}). In the present study we present supportive evidence that these post-pulses affect twitch tension to a large degree by their actions on the Na–Ca exchange mechanism. The effects of the slowly inactivating Ca^{2+} current can be discounted for the most part by the protocols used.

METHODS

The methods for measuring twitch tension and a^i_{Na} in voltage-clamped sheep Purkinje fibres have been previously described in detail elsewhere (Brill & Wasserstrom, 1986).

Preparations and solutions

Free-running Purkinje fibres were dissected from sheep hearts obtained from a slaughterhouse. Unbranched fibres less than 200 μm in diameter were used and were shortened to less than 2 mm in length by tying off the ends with silk suture. The fibres were attached to a photo-electric force transducer (TIL 138) at one end and secured to the tissue chamber floor with a pin at the other end. The preparations were stretched to approximately 140% of their resting length to achieve maximum base-line twitch tension. The fibres were superfused with Tyrode solution at 37 °C which was composed of (in mM): NaCl, 137; KCl, 5·4; $NaHCO_3$, 22; $MgCl_2$, 1; NaH_2PO_4, 2·4; $CaCl_2$, 1·8; dextrose, 5·5. The solution was bubbled with a 95% O_2–5% CO_2 gas mixture and had a pH between 7·2 and 7·3. Experiments performed in 0 Na^+ used solutions containing (in mM): Tris Cl (or TMA Cl or choline Cl), 152; KCl, 3; KH_2HPO_4, 2·4; $MgCl_2$, 1; $CaCl_2$ 1·8; HEPES (titrated with TrisOH), 20; dextrose, 5·5. The 0 Na^+ solutions had a pH between 7·2 and 7·3 and had osmolalities similar to the normal Tyrode solution (approximately 335–345 mosM). Extracellular Na^+ substitution was accomplished by first exposing the preparations for 5–10 min to 0 Na^+ containing solutions that were nominally Ca^{2+}-free followed by exposure to 0 Na^+ solutions with 1·8 mM-$CaCl_2$. Most experiments were performed using TrisCl as the Na^+ substitute because the fibres tolerated this replacement solution best. One experiment was performed with very low Na^+ solution (2 mM) using sucrose.

Measurement of a^i_{Na}

Measurements of a^i_{Na} were obtained using Na^+-sensitive micro-electrodes (Na^+ i.s.e.) prepared as previously described (Brill & Wasserstrom, 1986). Each electrode was calibrated both before and after each experiment using solutions with NaCl concentrations of 1, 3, 10, 30 and 100 mM with a balance of KCl to maintain a constant ionic strength of 300 mM. Electrodes used in experiments had slopes greater than 48 mV between 10 and 100 mM-Na^+. The response times of the electrodes were reduced to generally less than 1·0 s by capacitance compensation with a WPI d.c. amplifier (FC23B-A). Na^+ concentrations were calculated from a program imposing a best fit of the calibration data to the Nicolsky equation using an empirically verified Na^+/K^+ selectivity coefficient of 0·025 (program supplied courtesy of Dr Ole Sejersted). The correlation coefficients always exceeded 0·99 and the program-derived values for a^i_{Na} corresponded closely (generally $\pm$0·1 mM) with determinations from the calibration curve plotted by hand.

Voltage-clamp technique and protocols

The two-micro-electrode voltage-clamp technique was used as described in detail previously (Brill & Wasserstrom, 1986). Fibres were kept at a holding potential of -80 to -70 mV and standard pulses to 0 mV for 200 ms were applied at 0·25 Hz. This standard pulse was used in order to provide a uniform stimulus to contraction, presumably by the transient Ca^{2+} current. It was maintained for 200 ms which was long after the time of peak tension. Test pulses (post-pulses) for 1·8 s of varying amplitudes were applied at the same frequency at the end of the standard 200 ms pulses. Fibres therefore were held at the holding potential for a 2 s period followed by a 200 ms standard pulse

and a 1·8 s post-pulse. Intracellular Na^+ activity was measured at the end of the 2 s period between clamp steps, at which point the Na^+ i.s.e. signal had completely settled.

Data acquisition and analysis

Data were recorded on magnetic tape and Gould Brush 220 chart recorders during the experiments. Subsequently, data from magnetic tape were displayed on a Textronix 5113 storage oscilloscope and photographed for illustration purposes. All grouped data are presented as mean ± S.E. of mean.

Modelling

Mathematical modelling was done on an IBM-AT computer utilizing a simulation control program (SCOP, National Biomedical Simulation Resource, Duke University, Durham, NC, U.S.A.).

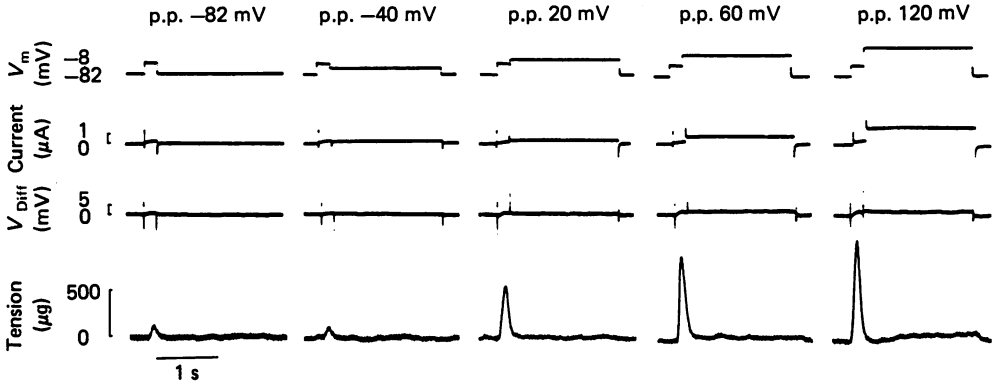

Fig. 1. The effects of post-pulse (p.p.) voltage on membrane voltage (top traces), current (upper middle traces), difference potential (lower middle traces), and twitch tension (bottom traces). Maximum voltage difference between voltage recording and independent electrodes was less than 4 mV (+120 mV panel). Twitch tension fell at post-pulses of −40 mV and then rose with progressively positive post-pulses. Note tonic tension in the +60 mV and +120 mV panels. Holding potential was −82 mV with standard pulses to −8 mV for 200 ms; depolarization frequency was 0·25 Hz.

RESULTS

Effects of post-pulses on twitch tension and demonstration of voltage control

Most experiments were performed using the protocol illustrated in Fig. 1. The fibre was maintained at a holding potential of −82 mV and standard pulses to −8 mV were applied for 200 ms at 0·25 Hz. Test pulses (post-pulses) of 1·8 s were delivered at the end of the standard pulses. In general the post-pulse potential was increased from the holding potential to more positive potentials in increments of 10–20 mV. At each post-pulse potential twitch tension was measured after a period of stimulation for 2–4 min, when tension had attained a new steady state.

In this illustrative experiment an independent electrode was impaled 250 μm from a standard voltage recording electrode connected to the feed-back circuit for voltage clamping. The difference in potential between the two recording sites (V_{diff}) represents any deviation from homogeneous voltage control. As demonstrated in Fig. 1 voltage control was excellent with a maximum voltage difference of only 3 mV at post-pulses of +120 mV (over-all voltage change of 200 mV).

In the experiment shown in Fig. 1 twitch tension was 158 μg in the control state when no post-pulse was applied (post-pulse potential equal to holding potential, −82 mV). When post-pulses to −40 mV were used, tension fell 26% to 117 μg. At a post-pulse potential of +20 mV tension rose to 566 μg, 258% above base line; at +60 mV tension rose to 947 μg. Each step to more positive post-pulses up to +120 mV resulted in greater tension, with steady-state tension values at +120 mV for twitch of 1104 μg (599% above control). A small amount of tonic tension is also apparent at post-pulses above +20 mV, although the induction of tonic tension was rarely seen in these experiments.

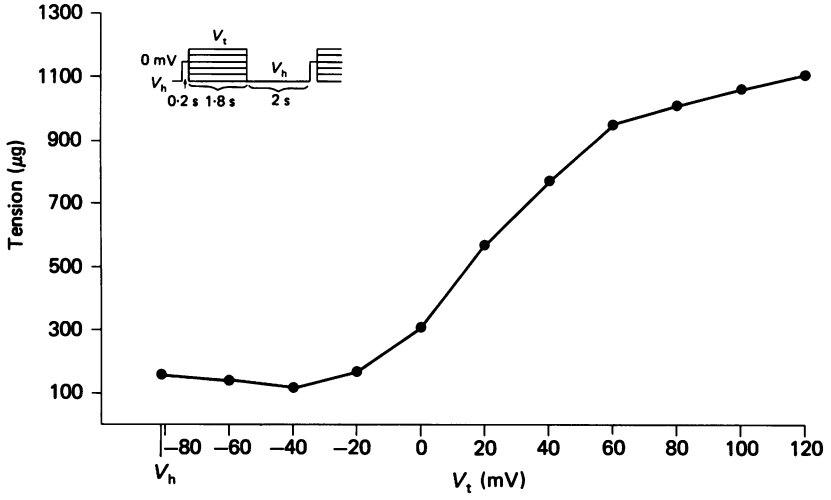

Fig. 2. The relation of steady-state twitch tension to post-pulse potential (V_t) from the experiment shown in Fig. 1. Above −20 mV tension increased with each 20 mV increment in post-pulse voltage.

In Fig. 2 the steady-state tension at various post-pulse potentials (V_t) is displayed graphically for the experiment shown in Fig. 1. Between −80 and −40 mV tension progressively declined reaching a nadir at −40 mV. At more positive post-pulses there was marked augmentation of tension, which continued to rise even at potentials of +120 mV. At these very positive potentials any effects of voltage on Ca^{2+} currents should be negligible as the reversal potentials for $I_{Ca,s}$ and $I_{Ca,f}$ are approached or exceeded.

In Fig. 3 the cumulative results are shown for a total of twelve different experiments using the same protocol as that illustrated in Fig. 1. Tension was normalized to the control tension value obtained at post-pulses (V_t) equal to the holding potential. The appearance of a nadir in tension at post-pulses of approximately −40 to −30 mV and the continued increase in tension compared to control at potentials above 0 mV were findings that were reproducible in multiple experiments.

The protocol for the experiment in Fig. 1 used a slow frequency of clamp step depolarization and prolonged post-pulse duration in order to exaggerate any voltage

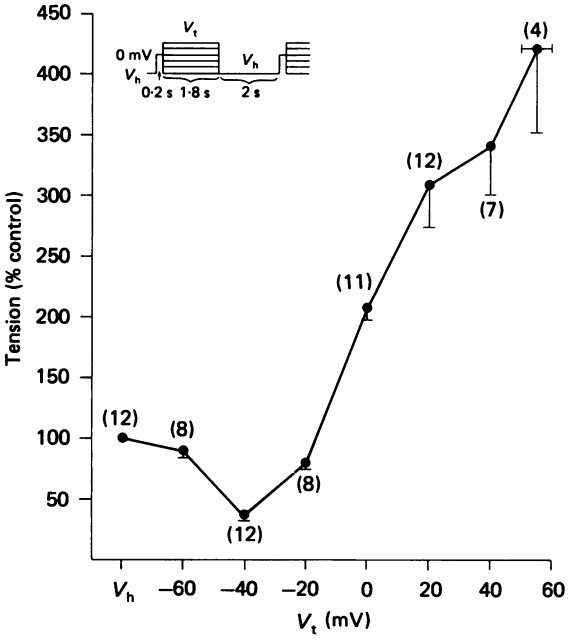

Fig. 3. Cumulative post-pulse voltage (V_t)–tension relation for a total of twelve experiments. Tension is expressed as the percentage of control tension obtained without post-pulse (V_t equal to V_h). Mean values are plotted for each data point ± S.E. of mean; n is indicated in parentheses.

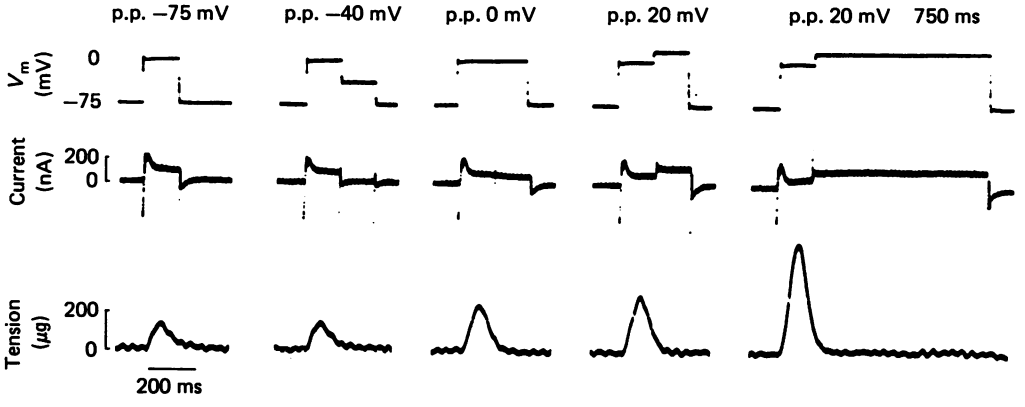

Fig. 4. The effects of post-pulse (p.p.) voltage of 150 ms in duration on voltage (top traces), current (middle traces), and twitch tension (bottom traces). Twitch tension at post-pulse voltages of 0 and +20 mV was elevated compared to control (p.p. −75 mV). When post-pulse was lengthened to 750 ms (p.p. 20 mV 750 ms), prominent augmentation of twitch was found. Holding potential was −75 mV with standard pulses to 0 mV for 150 ms; depolarization frequency was 0·5 Hz.

effects on tension and a^i_{Na} (see below). In Fig. 4 conditions were modified to demonstrate that post-pulses that more closely approximate physiological conditions can have a positive inotropic effect. The holding potential (V_h) in this experiment was −75 mV and standard pulses to 0 mV for 150 ms were given at a frequency of 0·5 Hz. Post-pulses to V_h, −40 mV, 0 mV and +20 mV were then applied for 150 ms. Post-pulses positive to −40 mV increased twitch tension from 136 μg at control to 244 μg at 0 mV and 271 μg at +20 mV representing 79 and 99 % increases relative to control. These changes in tension were modest compared to the rise in tension produced by longer post-pulses, such as the 750 ms post-pulse to +20 mV (far right panel, Fig. 4), that produced a twitch of 579 μg (326 % over control). Thus, the voltage effects on tension observed with long depolarizing post-pulses are also present, but are less pronounced when a shorter duration of depolarization is examined.

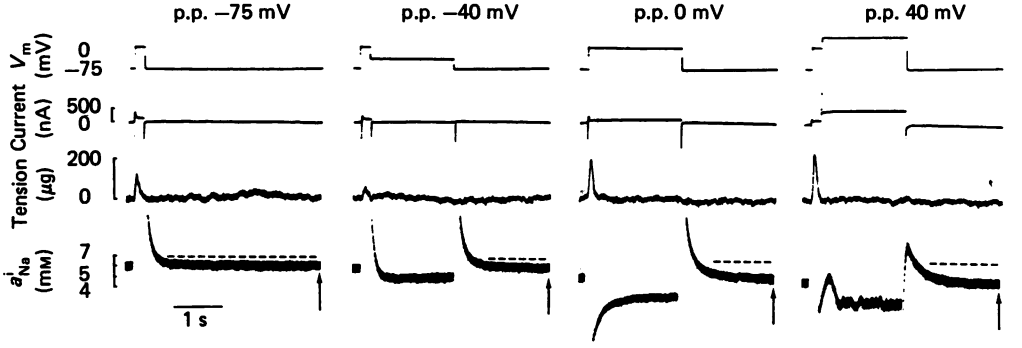

Fig. 5. Effects of post-pulse (p.p.) voltage on a^i_{Na} (bottom traces) and twitch tension (lower middle traces). Voltage and current are displayed in the top and upper middle traces respectively. a^i_{Na} was measured at the end of the 2 s period at V_h (indicated by arrows). a^i_{Na} declined with progressively positive post-pulses. Holding potential was −75 mV with standard pulses to 0 mV for 200 ms; depolarization frequency was 0·25 Hz.

Effects of post-pulses on a^i_{Na}

In the experiment displayed in Fig. 5, the holding potential was −75 mV and standard pulses to 0 mV for 200 ms were applied at 0·25 Hz. Post-pulses of 1·8 s were appended to the standard pulses. In this experiment a Na^+ i.s.e. was impaled within 100 μm of the voltage recording electrode. a^i_{Na} (bottom panel) was measured at the end of the 2 s period at V_h between voltage pulses (indicated by arrows in Fig. 5). As shown in this Figure, the recording of a^i_{Na} was stable at the end of this 2 s period.

The record for a^i_{Na} shows a small deviation during the post-pulses (see especially the +40 mV panel) compared to the tracing at V_h. This deviation in the a^i_{Na} record represents either shunting through the Na^+ i.s.e. that may vary in a potential-dependent manner or inhomogenous voltage control with a several millivolt voltage difference between the two sites during voltage pulses. Neither of these two types of difficulties with a^i_{Na} recordings during post-pulses is of importance since a^i_{Na} was measured at V_h where these sources of error are either consistent or absent. Eisner, Lederer & Vaughan-Jones (1981*a*) also found changes in their a^i_{Na} record during

depolarizing voltage-clamp pulses which they ascribed to voltage non-uniformity.

Fig. 5 shows that a^i_{Na} was lower with more positive post-pulses. During control (no post-pulse) and at −40 mV, a^i_{Na} was 6·2 mM; a^i_{Na} declined to 5·4 mM at 0 mV and 4·7 mM at +40 mV. Typical changes in twitch tension were also produced by post-pulses to 0 mV or higher.

These changes in a^i_{Na} with positive post-pulses were found in multiple experiments. This is illustrated in Fig. 6, which displays the cumulative results of six experiments examining the effects of post-pulses on a^i_{Na}. Changes in a^i_{Na} from control (no

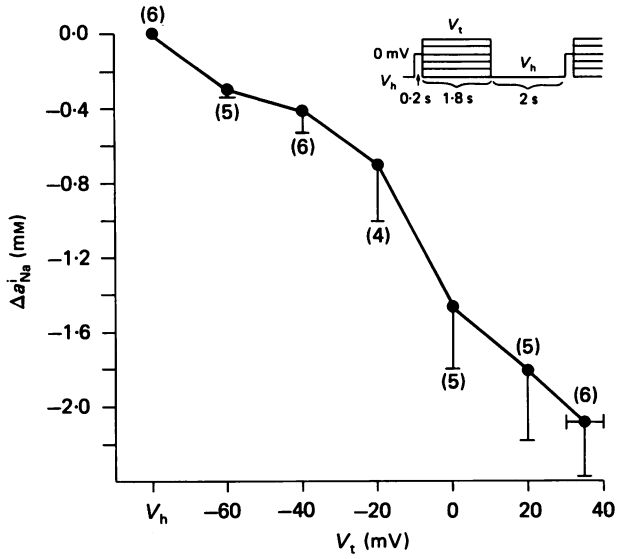

Fig. 6. Cumulative post-pulse voltage (V_t)–a^i_{Na} relation for a total of six experiments. a^i_{Na} is expressed as absolute change from control value for a^i_{Na} without post-pulse (V_t equal to V_h). Each point represents mean value ± S.E. of mean; n is indicated in parentheses.

post-pulse) *versus* post-pulse potential (V_t) are shown graphically. Under these experimental conditions, a^i_{Na} decreased at more positive post-pulse potentials. These experiments are the first demonstration of an inverse relation between a^i_{Na} and twitch tension, when voltage pulse amplitude is used to alter tension and a^i_{Na}. It is important to note that the changes in a^i_{Na} and tension with positive post-pulses were also present when the sequence of post-pulses began with the most positive level, followed by progressively more negative post-pulses. The effects on a^i_{Na} and tension (including the nadir in tension) were also present at depolarization frequencies of 0·2, 0·17 and 0·1 Hz.

Effects of tetrodotoxin and nitrendipine on changes in a^i_{Na} induced by post-pulses

The decrease in a^i_{Na} that occurs with progressively positive post-pulses may be the result of the reduced driving force for Na^+ entry through Na^+ channels as membrane potential approaches the reversal potential for Na^+. To test whether the decline in a^i_{Na} represents reduced leak or entry through Na^+ channels, the effect of tetrodotoxin (TTX) on the pattern of changes in tension and a^i_{Na} was studied with the usual

post-pulse protocol. In the experiment displayed in Fig. 7 the holding potential was −72 mV and standard pulses to 0 mV for 200 ms were applied at 0·25 Hz. The effects of post-pulses of 1·8 s on tension and a^i_{Na} are displayed in the left-hand panel. Typical changes in a^i_{Na} and tension are produced with a fall in the former and rise in the latter at positive potentials. After exposure to 100 μM-TTX base-line twitch tension without post-pulse was reduced from 260 μg (control panel) to 120 μg (right panel).

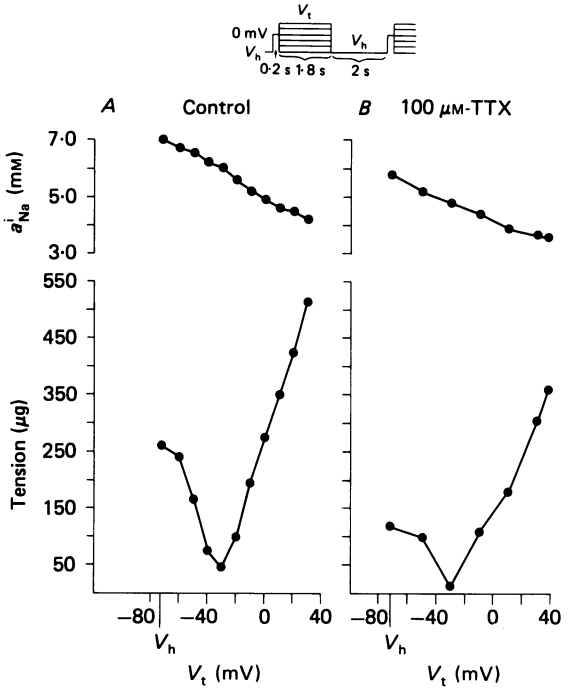

Fig. 7. Effects of 100 μM-TTX on relation of post-pulse voltage (V_t) to a^i_{Na} (upper graphs) and twitch tension (lower graphs). With control solution (left panel) typical changes in a^i_{Na} and twitch tension were present. After exposure to 100 μM-TTX (right panel) base-line twitch tension and a^i_{Na} without post-pulse (V_t equal to V_h) declined compared to control. Although absolute values were less for a^i_{Na} and tension with TTX, their over-all relation to post-pulse voltage was unchanged. Holding potential was −72 mV with standard pulses to 0 mV for 200 ms; depolarization frequency was 0·25 Hz.

Subsequently, progressively more-positive post-pulses were delivered. Tension reached a nadir at −30 mV and then increased with more-positive pulses to a maximum of 358 μg at +40 mV (198% increase from control TTX).

Base-line a^i_{Na} without post-pulse in 100 μM-TTX was 5·8 mM compared to 7·0 mM under control conditions. In the presence of TTX, a^i_{Na} still decreased with positive increments in post-pulse voltage to a minimum of 3·6 mM at +38 mV. At this concentration of TTX any Na^+ flux through Na^+ channels during the post-pulses should be virtually eliminated. The persistent decline in a^i_{Na} may be the result of another process, such as the Na–Ca exchange mechanism directed towards net Na^+ loss and net Ca^{2+} gain. The reduction in a^i_{Na} may also be due to a small fraction

of unblocked Na^+ channels, leak through non-Na^+ channels, or some other voltage-dependent Na^+ efflux mechanism (e.g. the Na pump or Na–H exchange).

The prominent augmentation of tension with positive post-pulses probably reflects increased Ca^{2+} loading of cells as opposed to changes in the stimulus for Ca^{2+} release, which should be invariant in view of the constant standard 200 ms pulse. Increased Ca^{2+} influx during the post-pulse may occur via a slowly inactivating Ca^{2+} current which is not yet turned off after 200 ms of depolarization. Although there are no highly specific blockers of $I_{Ca,s}$ that spare $I_{Ca,f}$ and permit contraction, a recent report (Bean, 1985) indicates that nitrendipine in 1–3 μM concentrations predominantly inhibits $I_{Ca,s}$ with little effect on $I_{Ca,f}$.

When the post-pulse voltage–tension relationship was examined after exposure to 1 μM-nitrendipine, post-pulses positive to 0 mV substantially increased tension compared to base-line tension (post-pulse at V_h). Although nitrendipine reduced the absolute value of tension at each potential compared to control tension without nitrendipine, the Ca^{2+} channel blocker did not alter the over-all relationship between tension and post-pulse potential including the nadir at −40 mV.

Background experiments in Na^+-free solutions

All of the above experiments are consistent with the hypothesis that a voltage-sensitive Na–Ca exchange mechanism may underlie the powerful positive inotropic effect observed with positive post-pulses. The voltage profile of the positive inotropic response and its insensitivity to nitrendipine blockade argue against Ca^{2+} loading via $I_{Ca,s}$. To test the dependence of the tension response to positive post-pulses on Na–Ca exchange, the effects of Na^+ removal from external solutions were examined.

Na^+ replacement in the external solutions not only would inhibit Na–Ca exchange, but also could lead to intracellular Ca^{2+} overload during Na^+ removal or to other perturbations of the internal milieu including acidification. The experiment displayed in Fig. 8 was designed to determine whether or not appropriate Ca^{2+} channel behaviour would persist in Na^+-free solutions. The fibre was initially voltage clamped in normal Tyrode solution; subsequently, voltage control was terminated and the solution was changed to a Tris-containing substitution solution without Ca^{2+} for 5 min. This was followed by exposure to Tris solution with 1·8 mM-$CaCl_2$ (see Methods); at this point voltage clamping was resumed. As shown in the control panel the fibre was held at −70 mV and pulses to −10 mV for 200 ms were applied at 0·5 Hz. Substantial net outward current was required during the depolarizing pulses. Twitch tension in 0 Na^+ was comparable to that generated in normal Tyrode solution.

A 3 min exposure to 10^{-6} M-Bay K 8644, a Ca^{2+} channel agonist with positive inotropic properties, caused a marked increase in twitch tension from 713 μg during control to 1260 μg. After a 12 min wash-out period, some reversal of the Bay K 8644 effect was observed. The inorganic Ca^{2+} channel blocker, $CdCl_2$ (0·5 mM), was then added to the Na^+-free superfusate. Twitch tension declined from 902 μg during the first wash-out to 152 μg in the presence of $CdCl_2$. Wash-out of the $CdCl_2$ resulted in partial recovery of tension to 424 μg. The current traces for this experiment show that a superimposed inward current appeared during exposure to Bay K 8644. This current presumably represents a Ca^{2+} current which subsequently was abolished during treatment with $CdCl_2$. The appropriate modulation of twitch tension with

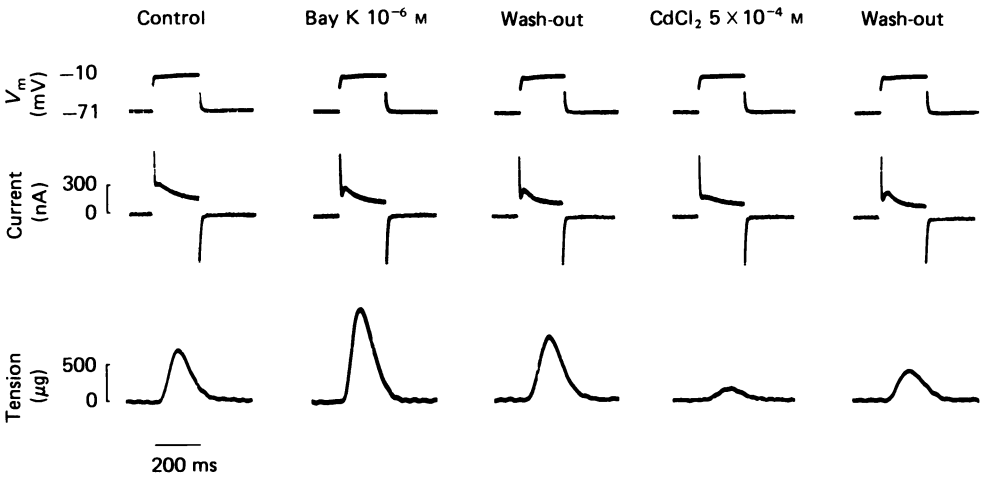

Fig. 8. Effects of Ca^{2+} channel modulating drugs on twitch tension in Na^+-free, Tris solution. Voltage (top traces), current (middle traces), and tension (bottom traces) are displayed. After exposure to 1 μM-Bay K 8644, a substantial increase in twitch tension occurred. Following partial wash-out of Bay K 8644 effect, exposure to 0·5 mM-$CdCl_2$ almost abolished tension. Far right panel shows some recovery from effects of $CdCl_2$ during wash-out. Holding potential was −71 mV with pulses to −10 mV at 0·5 Hz.

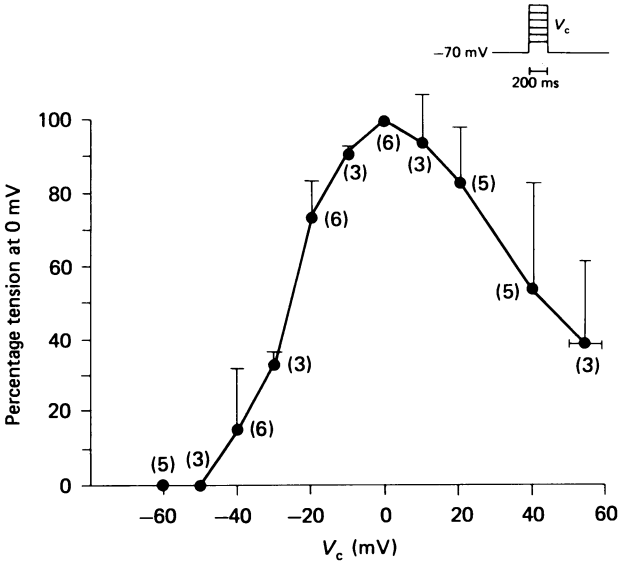

Fig. 9. Cumulative voltage–tension relation for a total of six experiments in 0 Na^+ solutions. Tension is normalized to the value obtained at 0 mV for each experiment where peak tension generally was present. Depolarizing pulses (V_c) 200 ms in duration of varying amplitude were delivered at a frequency of 0·5 Hz. Results are expressed as mean values ± S.E. of mean; n is indicated in parentheses.

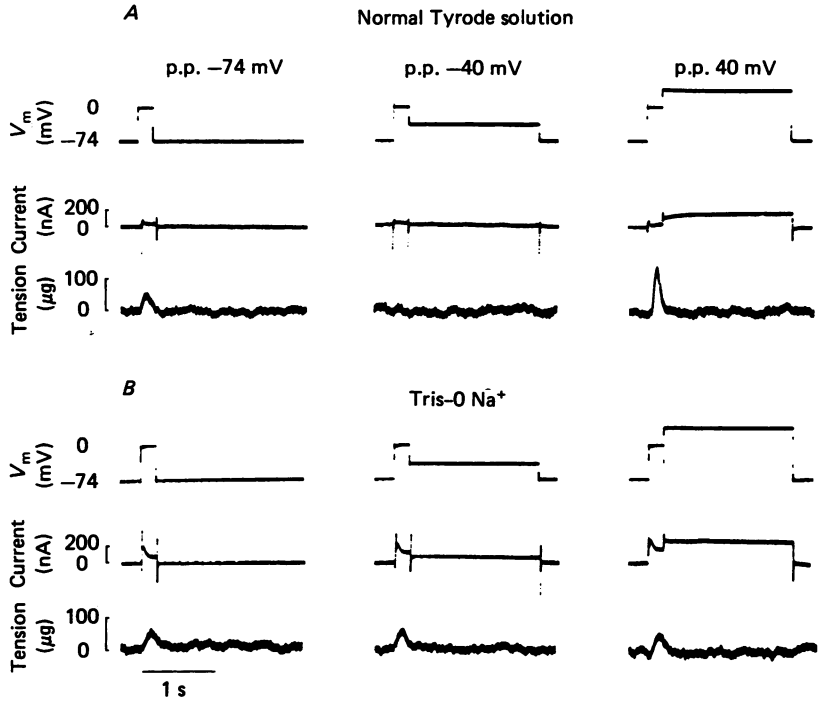

Fig. 10. Effects of Na^+ removal on post-pulse–tension relation. In *A* the effects of post-pulses (p.p.) to −74 mV (V_h), −40 mV, and +40 mV on voltage (top traces), current (middle traces), and tension (bottom traces) are displayed. Typical nadir at −40 mV and augmentation of tension at +40 mV were found. In *B* the same protocol was performed with post-pulses to −74 mV (V_h), −40 mV, and +40 mV in Na^+-free, Tris solution. The effects on voltage, current, and tension (top, middle, and bottom traces respectively) are shown. Tension response to the different post-pulses was essentially voltage independent. Holding potential was −74 mV with standard pulses to 0 mV for 200 ms; depolarization frequency was 0·25 Hz. There is some distortion artifact in the current records related to the transfer of data to and from magnetic tape.

Ca^{2+} channel agonists and blockers suggests that the Na^+-free system is acceptable for testing the effects of Na–Ca exchange inhibition. However, it should be noted that the magnitude of the response to Bay K 8644 was not uniform and that isoprenaline in pilot experiments failed to augment contractility.

The voltage–tension relation in 0 Na^+ solutions was also examined and the results of a total of six experiments are shown in Fig. 9. Fibres were held at approximately −70 mV. Voltage pulses to various clamp potentials (V_c) were applied for 200 ms at 0·5 Hz and steady-state tension was measured. V_c was increased in 10 or 20 mV increments from −60 mV. The values for tension displayed for Fig. 9 are normalized to the tension achieved at 0 mV where peak tension was generally measured. The first appearance of tension occurred at −40 mV. This corresponds with previous observations in normal solutions and represents the activation of I_{si} (Beeler & Reuter, 1970). The novel observation in 0 Na^+ solutions is the decline in tension at clamp potentials above 0 mV, a pattern of tension development that parallels the current–

voltage relation for I_{si}, but markedly differs from the continued rise in tension seen in Na^+-containing solutions (Gibbons & Fozzard, 1975).

Tension response to post-pulses in Na^+-free solutions

Fig. 10 shows the results of an experiment in which the typical post-pulse protocol was performed in normal Tyrode solution and then in a Tris-containing, Na^+-free solution. The fibre was maintained at a holding potential of −74 mV and standard pulses to 0 mV were delivered for 200 ms at 0·25 Hz. Post-pulses of 1·8 s were appended to the standard pulses. In Fig. 10*A* the effects of three different post-pulse potentials are displayed. Base-line tension without post-pulse (−74 mV) was 42 μg;

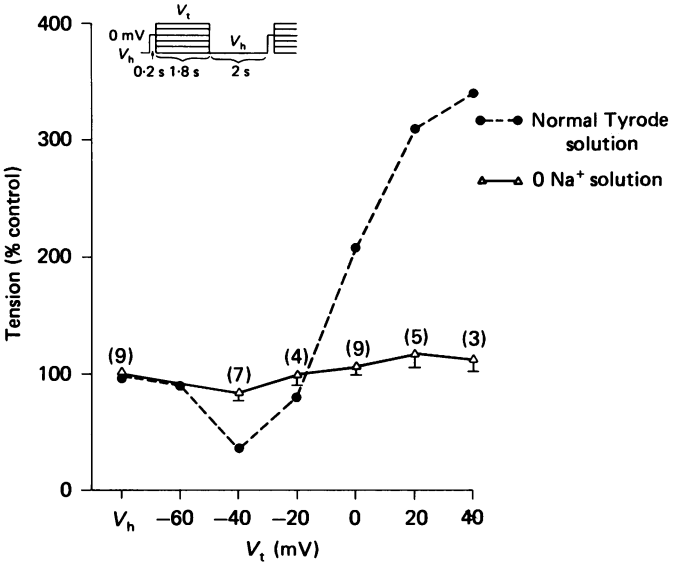

Fig. 11. Cumulative post-pulse voltage (V_t)–tension relation in 0 Na^+ solutions for a total of nine experiments. Tension is normalized to the value obtained without post-pulse (V_t equal to V_h). Results in 0 Na^+ are displayed by the continuous line and are expressed as mean values ± S.E. of mean; n for each point is indicated in parentheses. Post-pulse voltage–tension relation in normal Na^+ solutions from Fig. 3 is shown by the dashed line.

tension was undetectable with post-pulses to −40 mV, representing the typical nadir seen in other experiments. Post-pulses to +40 mV increased tension to 154 μg (267 % increase compared to base line).

After Na^+-free Tris solution was substituted for normal Tyrode solution (Fig. 10*B*), the base-line tension in the absence of a post-pulse (−74 mV) was comparable to that generated in normal solutions, 46 μg. When post-pulses to −40 or +40 mV were applied, the tensions developed were 42 and 52 μg, respectively. When the full range of post-pulse potentials was examined, the tension response was flat compared to the typical biphasic profile seen in normal solutions. Potentiation of contraction at positive voltages was abolished and the nadir in tension at −40 mV was not seen.

Fig. 11 demonstrates the post-pulse voltage–tension profile for a total of nine experiments examining the post-pulse response in Na^+-free solutions. Tension values

were normalized to the value obtained when no post-pulse (V_t equal to V_h) was applied. In 0 Na^+ solutions (continuous line) there was no augmentation of tension with positive post-pulse voltages. The nadir at −40 mV also was not prominent. The cumulative profile in normal solutions demonstrating the nadir at −40 mV and marked potentiation of twitch at positive post-pulses (from Fig. 3) is superimposed (dashed line) for comparison.

DISCUSSION

In the present study, we have developed a protocol that produces remarkable increases in twitch tension through the application of prolonged positive post-pulses at the end of a constant standard pulse. In addition, a^i_{Na} declined coincident with the rise in tension with positive post-pulses. This inversion of the typical a^i_{Na}–tension relation is unusual and has been described only once previously (Boyett & Hart, 1986). We interpret these results as evidence that the regulation of twitch tension under these conditions is dominated by voltage-sensitive Na–Ca exchange, which promotes net Ca^{2+} influx and net Na^+ efflux during positive pulses. Although a role for voltage-dependent Na–Ca exchange in the control of tonic tension has been shown in frog heart (Horackova & Vassort, 1979), this study presents evidence for such a voltage-dependent regulatory function for Na–Ca exchange with regard to twitch tension in mammalian heart muscle.

Implications of post-pulse voltage–tension relation

By maintaining an invariable standard depolarizing pulse of 200 ms, voltage influences are kept constant during activation of the contractile apparatus. The duration of 200 ms was selected because previous work with sheep Purkinje fibres (Gibbons & Fozzard, 1971) has shown that clamp duration did not alter peak tension until the duration was reduced below the time to peak tension (less than 100 ms). The post-pulse therefore cannot affect excitation–contraction coupling directly by modulating internal Ca^{2+} release or the stimulus for release. Rather, post-pulses most likely exert their effect by altering Ca^{2+} loading conditions. Increased Ca^{2+} influx presumably leads to an increase in s.r. Ca^{2+} stores and subsequent release during contraction, as has been demonstrated in skinned Purkinje cells (Fabiato, 1985). An additional effect of post-pulses on activation/inactivation kinetics of ion channels is unlikely in view of the persistence of the post-pulse effect on twitch tension with a depolarization frequency of 0·1 Hz which provides an 8 s interval at V_h.

Aside from Na–Ca exchange, the major alternative candidate that could influence Ca^{2+} loading is the newly described, slowly inactivating component of the Ca^{2+} current, $I_{Ca,s}$. Analysis of $I_{Ca,s}$ in bull-frog atrial cells, guinea-pig ventricular cells, and canine atrial cells has produced one consistent finding: reduction of peak current at potentials above +20 mV (Hume & Giles, 1983; Lee *et al.* 1984; Bean, 1985). Bean (1985) found a reversal potential, E_r, at +70 mV when Ca^{2+} was the charge carrier. Lee *et al.* (1984) determined E_r to be 0 mV and Hume & Giles (1983) found E_r for I_{slow} ($I_{Ca,s}$) to be +30 mV. Therefore, this current should be diminished considerably or be absent at potentials above +40 mV. The post-pulse voltage–tension relation, however, indicates that prominent increases in tension continue to occur even at

progressively positive voltages. A slowly inactivating Ca^{2+} current should be small at +50 to +60 mV where a greater than 400% rise in tension compared to control was found (Fig. 3). Furthermore, the voltage–tension relation for the experiment of Figs. 1 and 2 is incompatible with a dominant role for $I_{Ca,s}$ which should have reversed below +120 mV where maximal positive inotropic effect was found.

Another argument against a dominant role for $I_{Ca,s}$ centres on the post-pulse voltage–tension response in the presence of nitrendipine. Bean (1985) found that 1–3 μM-nitrendipine minimally affected $I_{Ca,f}$ while profoundly reducing $I_{Ca,s}$ at positive clamp potentials. In the present work, nitrendipine did not affect the post-pulse-induced augmentation of tension at positive voltages and in particular at +40 mV where Bean found strong inhibition of $I_{Ca,s}$ by nitrendipine. It should be pointed out that Bean worked with a different species and tissue (canine atrial cells). In addition, in the present study base-line twitch was reduced with nitrendipine indicating some inhibition of Ca^{2+} currents during the standard pulse to 0 mV. It is not possible from these experiments to differentiate between an effect of nitrendipine on the trigger Ca^{2+} and an effect on the Ca^{2+} channels to alter Ca^{2+} loading. Nevertheless, it still is a pertinent finding that nitrendipine did not alter the post-pulse voltage–tension response.

The Na–Ca exchange mechanism, if voltage sensitive, could progressively increase net Ca^{2+} influx with higher post-pulses. Although a recent review by Eisner & Lederer (1985) questions whether the exchanger is electrogenic, there is abundant evidence that the stoicheiometry of the exchange mechanism involves the counter-transport of more than two Na^{+} per Ca^{2+} (Pitts, 1979; Sheu & Fozzard, 1982; Bridge & Bassingthwaite, 1983; Reeves & Hale, 1984). Accordingly, the exchanger may be electrogenic and voltage sensitive, if the coupling ratio, n, is greater than 2. The recent measurement of currents generated by Na–Ca exchange in guinea-pig myocyte preparations (Kimura, Noma & Irisawa, 1986; Mechmann & Pott, 1986) provides compelling evidence that the exchanger is electrogenic. Depending on membrane voltage and the electrochemical gradient for Na^{+} and Ca^{2+}, the exchanger could directly transport Ca^{2+} in or out of the cell (Mullins, 1979). A voltage-sensitive Na–Ca exchange mechanism, though, does not necessarily need to reverse directions in order for cells to gain Ca^{2+}. Rather, a decline in Na^{+}-dependent Ca^{2+} efflux at more positive potentials in conjunction with a background Ca^{2+} influx would be sufficient to cause net intracellular Ca^{2+} gain and loading. It is the effect of Na–Ca exchange upon the balance of Ca^{2+} flux, not the absolute direction of Na–Ca exchange, that most likely determines whether Na–Ca exchange augments or reduces contractile force.

An unusual feature of the post-pulse voltage–tension relation is the fall in tension with negative post-pulses in the −40 mV range. The persistence of this nadir in the presence of Na^{+} channel blockade and Ca^{2+} channel blockade indicates that the phenomenon does not rely on manipulation of these channels. Furthermore, the virtual disappearance of this phenomenon in Na^{+}-free solutions suggests that it may be attributable to a Na^{+}-dependent process, such as Na–Ca exchange. The complex pattern of changes in tension with post-pulse voltage may be an indirect indication of Ca^{2+} flux via Na–Ca exchange. A description of Na–Ca exchange using a symmetric Eyring barrier (Hansen, Gradmann, Sanders & Slayman, 1981) to model voltage-dependent transitions can be used to predict Ca^{2+} flux by a voltage-sensitive charged

carrier. Such a model was proposed by Eisner & Lederer (1985) who used it to predict the shape of the Ca^{2+} flux–voltage relation for electrogenic and electroneutral Na–Ca exchange mechanisms. Their six-state cyclical model had twelve rate constants; four rate constants to describe voltage-dependent transmembrane movements and eight rate constants to describe binding and unbinding of internal Na^+, internal Ca^{2+}, external Na^+ and external Ca^{2+}. Although the assignment of individual rate constants is empirical, the form of the model is reasonable and can serve as a starting point. This model is especially pertinent to the present work, because it allows predictions of relative Ca^{2+} flux under steady-state conditions over any voltage range of interest.

When the ionic concentrations used in the present work are substituted into the Eisner & Lederer (1985) model for an electrogenic exchanger, the voltage relation for predicted net Ca^{2+} flux relative to flux at the holding potential (approximately -70 mV) can be examined (Fig. 12). A coupling ratio of 3 was used in this modelling system and Na^+ concentration was varied in a voltage-dependent manner based on our experimental measurements. The rate constant governing the binding of intracellular Na^+ was reduced by a factor of 4; all other rate constants were left unchanged from the Eisner & Lederer (1985) model. The model demonstrates that the exchanger can account for relative Ca^{2+} loss at potentials near -40 mV, whilst relative Ca^{2+} gain can occur at potentials above 0 mV. This pattern of Ca^{2+} flux parallels the observed post-pulse voltage–tension relation in Fig. 3. Although this model is limited by the lack of a firm experimental basis for individual rate constants, it does demonstrate that a voltage-sensitive Na–Ca exchange mechanism can account for the shape of the voltage–tension relation.

Post-pulses of long duration were used in most experiments to maximize any possible voltage effects on voltage-sensitive Na–Ca exchange and especially any effects on a^i_{Na}. It is also an important finding that standard pulses and post-pulses of 150 ms (Fig. 4), which are within the physiological range of depolarization duration, can also modulate tension. Pharmacological and pathological alterations that substantially disturb the duration or plateau of the action potential may also affect contractility by similar means.

Changes in a^i_{Na} with post-pulse protocol

The fall in a^i_{Na} observed with progressively positive post-pulses is another demonstration of the dependence of a^i_{Na} on membrane potential. Previous studies have shown that alterations in holding potential of several minutes in duration produce changes in a^i_{Na} with depolarization lowering a^i_{Na} (Eisner *et al.* 1981*b*; January & Fozzard, 1984). The changes in a^i_{Na} are most easily explained by a reduction in the passive electrochemical gradient for Na^+ leak that occurs during positive post-pulses. However, the decline in a^i_{Na} found at a frequency of 0·1 Hz militates against a simple explanation invoking the voltage effect on the driving force for Na^+ leak. At this frequency the post-pulse occupies less than 20 % of the cycle, whilst the influence of V_h should be predominant. In addition, the persistence of the post-pulse effect on a^i_{Na} in the presence of 100 μM-TTX indicates that the decline in a^i_{Na} does not rely entirely on manipulation of Na^+ leak or flux through Na^+ channels. Previous studies have shown that steady-state a^i_{Na} decreases in the presence of TTX

(Deitmer & Ellis, 1980; January & Fozzard, 1984), suggesting that some Na^+ leak occurs via Na^+ channels. Na^+ leak through TTX-insensitive Na^+ channels, through Na–H exchange, or through other channels would be unaffected by TTX, but this amount of influx is probably minor. The Na^+ pump also contributes to the changes in a^{i}_{Na}, because the pump has been shown in single myocytes to be voltage dependent in the range of voltage tested in our experiments (Gadsby, Kimura & Noma, 1985). Changes in the electrochemical gradient for Na^+ leak probably play a role in regulating a^{i}_{Na} but not an exclusive one.

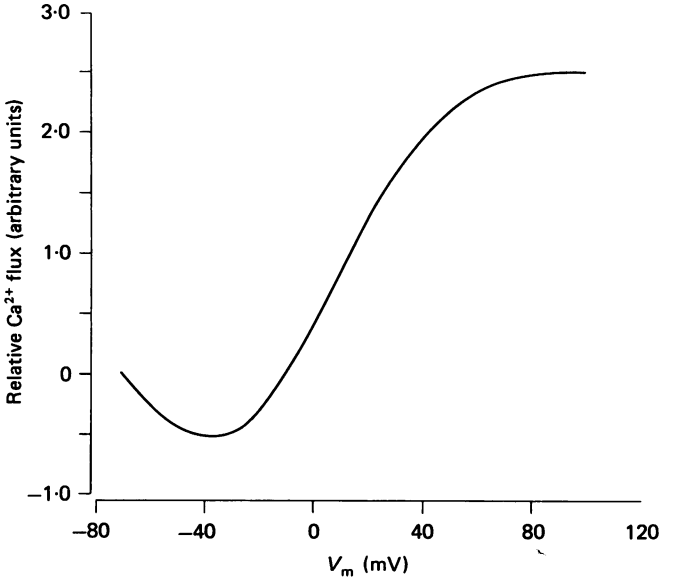

Fig. 12. The steady-state Ca^{2+} flux–voltage relation predicted by a model of Na–Ca exchange. Ca^{2+} flux has been normalized to the flux present at −70 mV. Relative Ca^{2+} influx is indicated in the positive direction.

It is also useful to consider in detail a role for the Na–Ca exchange mechanism in establishing the level of a^{i}_{Na}. If the influence of voltage on the exchanger is predominant during positive post-pulses, the Na^+ influx/Ca^{2+} efflux mode will be inhibited resulting in net Na^+ loss and net Ca^{2+} gain; or, the exchanger may even be directed in the Ca^{2+} influx/Na^+ efflux mode. Whenever the exchanger is responding primarily to voltage, a^{i}_{Na} secondarily will change. The corresponding increase in net Ca^{2+} influx would be reflected by increased twitch tension. On the other hand, in the voltage region near −40 mV, the effect of reduced Na^+ leak and consequent decline in a^{i}_{Na} may be paramount, so that the exchanger responds primarily to the increased Na^+ gradient for Na^+-dependent Ca^{2+} efflux. The result would be loss of intracellular Ca^{2+} and reduction in twitch tension. Ultimately, the over-all pattern of change for a^{i}_{Na} probably reflects the combined effects of changes in Na^+ leak and changes in Na^+ influx and efflux via Na–Ca exchange. The net influx of Ca^{2+} produced by the exchanger at positive post-pulses presumably is rapidly distributed to intracellular storage sites, such as the s.r. and mitochondria. As a result, tonic tension

was typically absent, probably because the systems responsible for intracellular Ca^{2+} storage were not yet saturated.

The profound increase in twitch tension associated with a reduction in a^i_{Na} is an exceptional finding in view of the typical positive correlation between a^i_{Na} and tension. Elevation of a^i_{Na} by Na^+ pump inhibition (Wasserstrom, Schwartz & Fozzard, 1983; Eisner, Lederer & Vaughan-Jones, 1984), frequency of stimulation (Cohen, Fozzard & Sheu, 1982) and treatment with veratridine (Brill & Wasserstrom, 1986) all have been associated with increased contractility. A brief report described a decline in a^i_{Na} during voltage-clamp trains in spite of increases in tension when pulse duration was lengthened (Boyett & Hart, 1986). The present study is the first description of an inverse a^i_{Na}–tension relation when a^i_{Na} is manipulated by changing the amplitude of voltage-clamp pulses. This finding can be explained by the actions of the Na–Ca exchange mechanism responding primarily to voltage influences. Rather than promoting Ca^{2+} efflux, the fall in a^i_{Na} may be a marker for voltage-mediated net Ca^{2+} influx. The observation of Boyett & Hart (1986) using a system varying pulse duration may in fact represent a similar effect of voltage on the exchanger with the longer duration pulses. Depending on the voltage-clamp protocol used, a^i_{Na} may be determined by the holding potential, clamp potential, relative amounts of time at each potential, frequency of depolarization, state of Na^+ channels, and activity of the Na^+ pump.

Effect of 0 Na^+ solutions on twitch tension

The complete suppression of the rise in tension induced with post-pulses by the use of Na^+-free solutions indicates that the phenomenon relies on a Na^+-dependent mechanism, presumably Na–Ca exchange. The major limitation of the 0 Na^+ experiments is the uncertainty regarding the integrity of the contractile processes in this unnatural environment. The responsiveness to Ca^{2+} channel manipulation, although reassuring, was inconsistent at times. The absence of response to isoprenaline could have been the result of alteration in receptor–agonist binding or in subsequent steps in the receptor–effector cascade related to the Na^+ substitution solutions. The inhibition of Na–H exchange could also produce acidification that would be deleterious. However, direct measurement of intracellular pH (pH_i) has shown only relatively minor intracellular acidification in 0 Na^+ (Ellis & MacLeod, 1983).

The major concern with a 0 Na^+ system is the possibility of Ca^{2+} overload. The use of nominally Ca^{2+}-free solution during Na^+ removal should have minimized Ca^{2+} loading. The inhibition of Na–Ca exchange should not necessarily lead to flooding of the cell with Ca^{2+}. Wendt & Langer (1977) using ion flux studies in rabbit ventricle found only a 28% reduction in the rate of Ca^{2+} efflux in 0 Na^+ compared to normal extracellular Na^+ solutions. Rasmussen, Bridge, Ishida & Barry (1985) found total Ca^{2+} content decreased in cultured chick embryo ventricular cells in Na^+-free solutions loaded with Ca^{2+} by caffeine treatment. Clearly, heart cells are able to defend themselves against Ca^{2+} overload in the absence of external Na^+ through a non-Na^+-dependent mechanism, most likely the sarcolemmal Ca^{2+}-ATPase (Caroni & Carafoli, 1980). Ca^{2+} loading in the presence of Na^+-free solutions would also be buffered by increased Ca^{2+} uptake by the sarcoplasmic reticulum and especially by

mitochondria which can accumulate large quantities of Ca^{2+} by forming hydroxyapatite precipitates (see Carafoli, 1985, for review). Most importantly, we did not observe any evidence of Ca^{2+} overload such as after-depolarization or the induction of the transient inward current, I_{ti}. Many preparations were viable for long periods of time and frequently exhibited normal mechanical function after restoration of external Na^{+}.

The steady-state voltage–tension relation in 0 Na^{+} demonstrated a descending limb at potentials above 0 mV (Fig. 9). the elimination of Na–Ca exchange in 0 Na^{+} may be responsible for the shape of the curve. The observation that the voltage–tension relation is influenced by external Na^{+} concentration was also made by New & Trautwein (1972) who found that tension declined with pulses positive to 0 mV when Na^{+} was reduced from 140 to 12 mM. Gibbons & Fozzard (1975) derived two different voltage–tension curves depending on the protocol used. When the first voltage pulse after a period of quiescence was examined, the relation was similar to our results in 0 Na^{+}. However, steady-state tension measured after ten or eleven pulses increased with more-positive clamp voltages. With a pulse duration of 300 ms in the Gibbons & Fozzard (1975) protocol there would be ample time for Na–Ca exchange to favour net Ca^{2+} gain at positive potentials. The Ca^{2+} loading action of the exchanger would require several pulses to be fully manifested. Hence, the influence of Na–Ca exchange on the voltage–tension relation would be negligible with the first contraction after a prolonged quiescent period, but would strongly affect steady-state tension.

Possible mechanisms for tension nadir with post-pulse protocol

As mentioned above, it is possible to model the Ca^{2+} fluxes in an electrogenic Na–Ca exchange system so that net Ca^{2+} loss occurs during negative post-pulses (Eisner & Lederer, 1985). Alternatively, the nadir may reflect the interaction of changes in voltage and a^{i}_{Na} on Na–Ca exchange. With post-pulses near −40 mV, a reduction in a^{i}_{Na} due to reduced Na^{+} leak would then increase the driving force for Na^{+}-dependent Ca^{2+} efflux; when post-pulse voltage is sufficiently positive, the exchanger will be dominated by voltage influences leading to net Ca^{2+} influx and secondary changes in a^{i}_{Na}.

The restitution properties of heart tissue may contribute to the tension nadir. In voltage-clamped sheep Purkinje fibres Lipsius, Fozzard & Gibbons (1982) found that conditioning pulses to −42 mV required a repolarization time of at least 3 s to restore twitch tension to control value. Control tension was determined by voltage pulses after a 90 s rest period; this is an important difference in protocol compared to the present study. Also, when a depolarization frequency of 0·1 Hz was used in the present work (8 s period at V_h), the nadir was still observed. Although the influence of restitution processes on the nadir cannot be established definitively, it certainly cannot be discounted. Finally, post-pulses in the −40 mV range may have some undetermined effect on the kinetics of the Ca^{2+} currents that requires longer than 8 s to recover.

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REFERENCES

BEAN, B. P. (1985). Two kinds of calcium channels in canine atrial cells. Differences in kinetics, selectivity, and pharmacology. *Journal of General Physiology* **86**, 1–30.

BEELER, G. W. & REUTER, H. (1970). The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. *Journal of Physiology* **207**, 211–229.

BOYETT, M. R. & HART, G. (1986). Dissociation between force and intracellular sodium activity (a^i_{Na}) during the force staircase in isolated sheep Purkinje fibres. *Journal of Physiology* **370**, 51*P*.

BRIDGE, J. H. B. & BASSINGTHWAITE, J. B. (1983). Uphill sodium transport driven by an inward calcium gradient in heart muscle. *Science* **219**, 178–180.

BRILL, D. M. & WASSERSTROM, J. A. (1986). Intracellular sodium and the positive inotropic effect of veratridine and cardiac glycoside in sheep Purkinje fibres. *Circulation Research* **58**, 109–119.

CARAFOLI, E. (1985). The homeostasis of calcium in heart cells. *Journal of Molecular and Cellular Cardiology* **17**, 203–212.

CARONI, P. & CARAFOLI, E. (1980). An ATP-dependent Ca^{2+}-pumping system in dog heart sarcolemma. *Nature* **283**, 765–767.

COHEN, C. J., FOZZARD, H. A. & SHEU, S.-S. (1982). Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circulation Research* **50**, 651–662.

DEITMER, J. W. & ELLIS, D. (1980). The intracellular sodium activity of sheep heart Purkinje fibres: effects of local anaesthetics and tetrodotoxin. *Journal of Physiology* **303**, 269–282.

EISNER, D. A. & LEDERER, W. J. (1985). Na–Ca exchange: stoichiometry and electrogenicity. *American Journal of Physiology* **248**, C189–202.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1981*a*). The dependence of sodium pumping and tension on intracellular sodium activity in voltage-clamped sheep Purkinje fibres. *Journal of Physiology* **317**, 163–187.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1981*b*). The effects of rubidium ions and membrane potential on the intracellular sodium ion activity of sheep Purkinje fibres. *Journal of Physiology* **317**, 189–205.

EISNER, D. A., LEDERER, W. J. & VAUGHAN-JONES, R. D. (1984). The quantitative relationship between twitch tension and intracellular sodium activity in sheep cardiac Purkinje fibres. *Journal of Physiology* **355**, 251–266.

ELLIS, D. & MACLEOD, K. T. (1983). Ca-dependent effects of extracellular Na removal on intracellular pH in isolated sheep heart Purkinje fibres. *Journal of Physiology* **342**, 54–55*P*.

FABIATO, A. (1983). Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *American Journal of Physiology* **245**, C1–14.

FABIATO, A. (1985). Simulated calcium current can both cause calcium loading in and trigger calcium release from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. *Journal of General Physiology* **85**, 291–320.

GADSBY, D. C., KIMURA, J. & NOMA, A. (1985). Voltage dependence of Na/K pump current in isolated heart cells. *Nature* **315**, 63–65.

GIBBONS, W. R. & FOZZARD, H. A. (1971). Voltage dependence and time dependence of contraction in sheep cardiac Purkinje fibers. *Circulation Research* **28**, 446–460.

GIBBONS, W. R. & FOZZARD, H. A. (1975). Relationships between voltage and tension in sheep cardiac Purkinje fibers. *Journal of General Physiology* **65**, 345–365.

HANSEN, U.-P., GRADMANN, D., SANDERS, D. & SLAYMAN, C. L. (1981). Interpretation of current–voltage relationships for 'active' ion transport systems: I. Steady state reaction-kinetic analysis of class-I mechanisms. *Journal of Membrane Biology* **63**, 165–190.

HORACKOVA, M. & VASSORT, G. (1979). Sodium–calcium exchange in regulation of cardiac contractility. Evidence for an electrogenic, voltage-dependent mechanism. *Journal of General Physiology* **73**, 403–424.

HUME, J. R. & GILES, W. (1983). Ionic currents in single isolated bullfrog atrial cells. *Journal of General Physiology* **83**, 153–194.

JANUARY, C. T. & FOZZARD, H. A. (1984). The effects of membrane potential, extracellular potassium, and tetrodotoxin on the intracellular sodium ion activity of sheep cardiac muscle. *Circulation Research* **54**, 652–665.

KIMURA, J., NOMA, A. & IRISAWA, H. (1986). Na–Ca exchange current in mammalian heart cells. *Nature* **319**, 596–597.

LEE, K. S., NOBLE, D., LEE, E. & SPINDLER, A. J. (1984). A new calcium current underlying the plateau of the cardiac action potential. *Proceedings of the Royal Society* B **223**, 35–48.

LIPSIUS, S. L., FOZZARD, H. A. & GIBBONS, W. R. (1982). Voltage and time dependence of restitution in heart. *American Journal of Physiology* **243**, H68–76.

MECHMANN, S. & POTT, L. (1986). Identification of Na–Ca exchange current in single cardiac myocytes. *Nature* **319**, 597–599.

MULLINS, L. J. (1979). The generation of electric currents in cardiac fibers by Na/Ca exchange. *American Journal of Physiology* **236**, C103–110.

NEW, W. & TRAUTWEIN, W. (1972). The ionic nature of slow inward current and its relation to contraction. *Pflügers Archiv* **334**, 24–38.

NOBLE, D. (1984). The surprising heart: a review of recent progress in cardiac electrophysiology. *Journal of Physiology* **353**, 1–50.

PITTS, B. J. R. (1979). Stoichiometry of sodium–calcium exchange in cardiac sarcolemmal vesicles. *Journal of Biological Chemistry* **254**, 6232–6235.

RASMUSSEN, C. A., BRIDGE, J. H. B., ISHIDA, H. & BARRY, W. H. (1985). Non-Na_o-dependent relaxation and Ca efflux in cultured chick embryo ventricular cells. *Circulation* **72**, suppl. III, 1301.

REEVES, J. P. & HALE, C. C. (1984). The stoichiometry of the cardiac sodium–calcium exchange system. *Journal of Biological Chemistry* **259**, 7733–7739.

REUTER, H. & SEITZ, N. (1968). The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *Journal of Physiology* **195**, 451–470.

SHEU, S.-S. & FOZZARD, H. A. (1982). Transmembrane Na^+ and Ca^{2+} electrochemical gradients in cardiac muscle and their relationship to force development. *Journal of General Physiology* **80**, 325–351.

WASSERSTROM, J. A., SCHWARTZ, D. J. & FOZZARD, H. A. (1983). Relation between intracellular sodium and twitch tension in sheep cardiac Purkinje strands exposed to cardiac glycosides. *Circulation Research* **52**, 697–705.

WENDT, I. R. & LANGER, G. A. (1977). The sodium–calcium relationship in mammalian myocardium: effect of sodium deficient perfusion on calcium fluxes. *Journal of Molecular and Cellular Cardiology* **9**, 551–564.